ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

VOLUME 71, ART. 3 PAGES 305-320

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TRANSPLANTATION OF FINS IN XIPHOPHORIN FISHES*

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Introduction

Held (1951) successfully interchanged the dorsal and anal fins of the platyfish *Xiphophorus maculatus*. He found that, soon after the transplantation from their normal position to another site, the fins sloughed off down to their bases; then, about four weeks later, some of the fins began to regenerate from their intact basal stumps. Regardless of their atypical position, the autotransplanted anal fins regenerated into anal fins and the transplanted dorsal fins regenerated into dorsal fins. If anal fin transplants made in females were exposed to androgenic substances, the reforming anal fins attained some features characteristic of the gonopodia of males, but the regenerating dorsal fins were not influenced by androgens. When Held attempted to transplant fins from one platyfish to another of its siblings, all the homotransplants failed. He attributed the failure to genetic differences between the donor and host in his platyfish stocks.

The Genetics Laboratory of the New York Zoological Society had a highly inbred stock of platyfish that had been developed by brother-sister matings for seventeen generations, actually since 1939. It also had other platyfish that had been inbred for fewer generations and still other fish that were not inbred at all. These stocks of platyfish seemed suitable for evaluating the role of genetic factors in the success or failure of fin transplantation. We anticipated that transplantations of fins from one platyfish to its siblings would succeed in the members of our most highly inbred stock and, perhaps, in some of the others. This is the report of our experiments.

Material and Methods

Terminology. An autotransplant denotes a transfer of tissues from their original site to another part of the body of the same individual.

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*Based on a thesis submitted by Klaus D. Kallman to the Graduate School of Arts and Sciences of New York University, in partial fulfillment of the requirements for the degree of Master of Science. The work described in this paper was aided by a grant from the National Cancer Institute, National Institutes of Health, Bethesda, Md., to the Genetics Laboratory of the Aquarium, New York Zoological Society, at the American Museum of Natural History, New York, N.Y.

An isotransplant represents a tissue transplant between a host and a donor that are members of the same highly inbred strain.

The term homotransplant refers to tissue transferred from one individual to another member of the same species, but not to members of the same highly inbred strain.

A heterotransplant involves a transfer of tissues between members representing different species or different species hybrids.

A successful transplant is one that is maintained in a living state indefinitely. A successful fin transplant may undergo initial and partial degeneration (sloughing), after which it regenerates and subsequently survives. Indications for success of a fin transplant could be noted, in our experiments, six to eight weeks after the transfer. Those that were intact and alive for eight weeks survived as long as the host animal. Some remained alive for as long as two years.

Soon after a fin has been transplanted, the distal portion usually begins to degenerate, but this degeneration, or sloughing, may stop at various levels. Eventually, regeneration of the transplanted fin stems from the base of the intact fin.

Genetic history of fishes. Various genetic stocks of *X. maculatus* (TABLE 1), which differed with regard to the degree of inbreeding, were selected.

TABLE 1

SUMMARY OF TRANSPLANTATION EXPERIMENTS

Pedigree		*Inbred*	*Type of*	*Fate*		
Host	*Donor*	*generations*	*transplant*	*Failures*	*Successes*	*Total*
30	30	17	Iso	0	14	14
163	163	6	Iso	2	11	13
Bh, gray*	Bh, gray*		Homo	3	1	4
Bh, red*	Bh, red*		Homo	4	0	4
492	492		Homo	4	1	5
500**	500**		Homo	2	9	11
501	500**		Homo	1	3	4
427	427		Homo	14	0	14
274	274		Homo	39	1	40
374	374		Homo	6	2	8
457	457		Homo	6	2	8

*Inbred for seven generations, but not by brother-sister matings.

**F_1 offspring of $Bh^7 \times 30^{16}$ (the 30^{16} parent inbred for 16 generations).

Strains 30^{17} and 163^6 originally came from the Rio Jamapa population. The former had been inbred by brother-sister matings for 17 generations; the latter, for only 6 generations.

The spotted platyfish of the Bh^7 strain were inbred in part for 7 generations, but not by brother-sister matings. One substrain was red, the other gray. Strain 492 was produced by mating members representing the two Bh^7 strains.

Fish of pedigree 500 were the F_1 offspring of crosses between red Bh^7 and 30^{16}; those of 501 were the F_1 offspring from matings of gray Bh^7 and 30^{16}. The fish of strains 274, 374, 427, and 457 were not inbred.

Technique. All stock fish were kept in conditioned aquarium water according to the methods described by Gordon (1950). Prior to the operation, the donor and the host were anesthetized in a dilute solution of Tricain Methanesulfonate (Sandoz's MS 222). After the fish had received their fin grafts they were placed in water containing 0.67 per cent household salt to inhibit infection. The technique for the removal of the fins and their implantation was similar to that described by Held.

The donor fish was placed in a dish and held by wet cotton pads in such a way that the fin to be excised was uppermost. Working under the dissecting microscope, all scales around the base of the dorsal or anal fin were removed with a pair of fine forceps. Then a longitudinal incision, approximately 0.5 mm. on either side of the first fin ray, was made with a fine dissecting scalpel. The scalpel was inserted at an oblique angle so that the blade was pushed underneath the fin until it reached the distal segments of the radials of the dorsal or anal fin (according to Rubin and Gordon, 1953, the radials of the anal fin suspensorium in adult females and immature fish consist of three segments: the proximal spinelike *actinosts*, the median *mesonosts*, and the distal *baseosts*; the baseosts articulate with the fin rays). The cut was made in a posterior direction along the whole basal length of one side of the dorsal or anal fin, severing all principal muscles; the same procedure was then repeated on the other side of the fin. The fin was then grasped near its anterior end with a pair of curved forceps, and the radials of the fin were gently freed. The whole fin was immediately placed into "amphibian" Ringer's solution and all loosely adhering tissue fragments were removed from it. When the dorsal fin was obtained from an animal larger than the host, the anterior and posterior end of the fin were trimmed to a suitable size. Trimming the transplanted fin did not reduce its chances for successful growth.

The host was placed in a dish, and its exposed head and gills were covered with wet cotton. During the operation the experimenter's left index finger pressed gently on the wet cotton to hold the fish in place. The scales were removed from the transplantation site; then a small dorso-ventral incision was made. The scalpel was withdrawn and then reinserted to open the incision to about three quarters of the length of the base of the excised fin, and to a depth determined by the length of the fin's radial elements. With a pair of forceps the fin was held in such a way that its radials pointed toward the prepared pocket. The mouth of the pocket was forced open by a gentle downward motion of the forceps; then, without releasing the pressure upon the pair of forceps, the basal portion of the slightly compressed fin was fitted into the pocket. After the operation the fish was placed in an aquarium in semidarkness where it was left undisturbed for at least forty-eight hours.

In fish only a few days old a different method for the removal and implantation of the dorsal and anal fins was used. A small donor fish was placed on its side on a wet glass plate. The dorsal or anal fin was removed with a razor blade by cutting slowly in an anterior-posterior direction about one half of a millimeter below the fin. The severed fin was immediately placed into Ringer's solution and the loose adhering tissue was removed with two sharp needles. A host fish was placed on smooth wet filter paper. With a fine glass needle a dorsoventral slit was made in the myotomes; the loose scales around the opening were removed. The base of the excised fin was gently pushed into the prepared slit. The very young host fish were kept in a glass container containing 500 cc. of Holtfreter's solution.

The best site for fin implantation was found to be in either side of the caudal peduncle just posterior to the anal fin. There, the myotomes are well developed and the incisions were sufficiently deep to receive the grafts neatly (FIGURE 1). In larger fish the operation was easier.

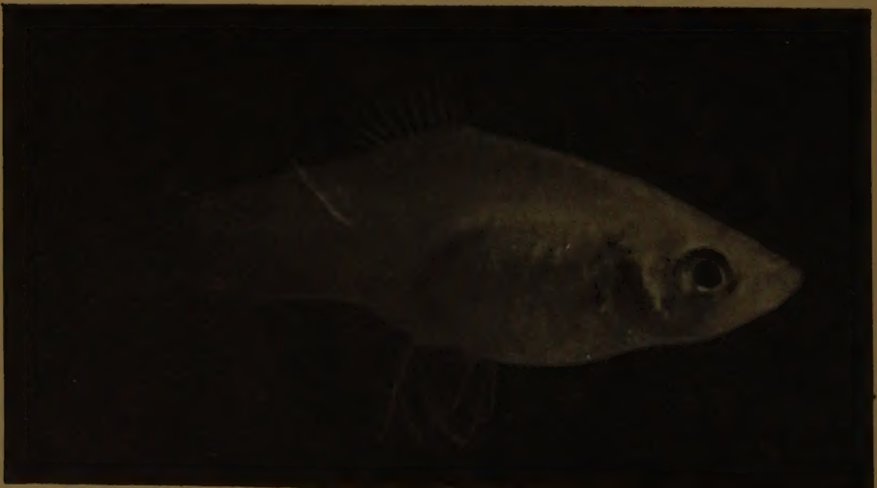

FIGURE 1. A live female platyfish of strain 30^{17} showing a successful isotransplant of an anal fin in the musculature of the caudal peduncle near the lateral line. Photo by Sam C. Dunton.

Histological examinations were made of normal fins, of those successfully transplanted, and of those in various stages of degeneration. Fish were fixed in either Bouin's picroformol or formalin and were sectioned at 5 μ; the sections were stained with Harris's hematoxylin and eosin or with Masson's trichrome.

Results

Isotransplants. Six females and 2 males of the highly inbred strain 30^{17} received dorsal fins from male donor fish; 6 additional adult females received anal fin grafts from immature females (TABLE 2, Nos. 1 to 14). Between the third and sixth day after the operation, hemorrhagic areas appeared in nearly all grafts. After the eighth or ninth days, in each of 12 host fish the distal margin of the fin disintegrated, and then the membranous tissue between the fin rays sloughed away down to the base of the fin. Histological examination revealed that many large phagocytes had accumulated around the base of the fin rays and had engulfed the cellular debris; elsewhere, free erythrocytes were present in large numbers (FIGURE 2).

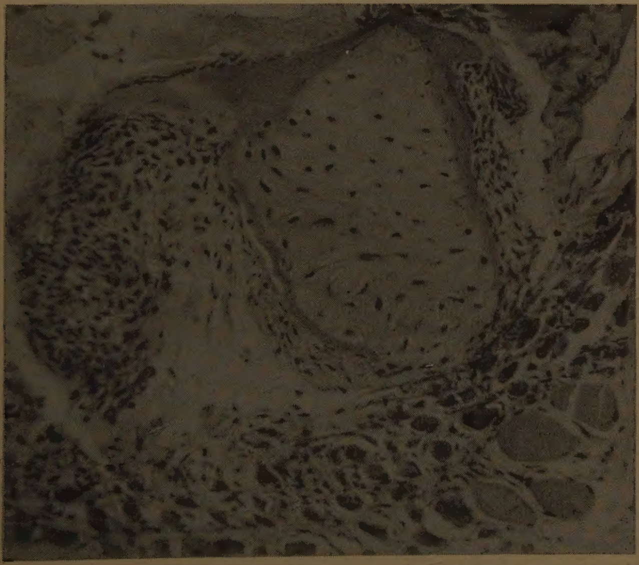

FIGURE 2. Cross section through an isotransplant of a dorsal fin in a platyfish of strain 30^{17} fixed and sectioned eight days after transplantation. Many phagocytic cells have accumulated in a band between a fin ray of the transplant and host myotomes. Very little cellular destruction or disorganization is apparent. Hematoxylin and eosin. ×215.

TABLE 2

HISTORIES OF TRANSPLANTED FINS IN ADULT PLATYFISH, *XIPHOPHORUS MACULATUS*

Pedigree	*Number*	*Sex*	*Fin*	*Sloughing in days*[*]	*Regeneration in days*[**]	*Survival in days*[†]
30^{17}	1 to 6	♀	Dorsal	8, 9	28	S
"	7	♂	Dorsal	none	—	S
"	8	♂	Dorsal	9	26	S
"	9 to 11	♀	Anal	8, 9	21 to 26	S
"	12, 13	♀	Anal	8, 9	21 to 26	S
"	14	♀	Anal	none	—	S
500	15, 16	♀	Anal	4, 5	none	12 to 14
"	17 to 20	♀	Dorsal	3 to 9	22	S
"	21, 22	♀	Dorsal	5	22	S
"	23 to 25	♀	Anal	4 to 7	28	S
501	26	♂	Anal	4	none	50
"	27 to 29	♂	Anal	4	21	S
Bh, red	30 to 32	♀	Dorsal	3, 7	none	11 to 13
"	33	♀	Dorsal	5	none	63
Bh, gray	34 to 36	♀	Dorsal	17 to 19	none	22
"	37	♀	Dorsal	none	—	S
492	38 to 41	♀	Dorsal	3 to 7	none	12 to 14
"	42	♀	Dorsal	6	24	S
163^{6}	43, 44	♀	Dorsal	3 to 5	none	9
"	45 to 48	♂	Dorsal	3 to 4	25	S
"	49 to 55	♀	Dorsal	3 to 4	25 to 30	S
427	56 to 69	♀, ♂	Both	3 to 7	none	7 to 9
274	70 to 91	♀	Both	3 to 9	none	5 to 14
374	92 to 97	♀	Both	3 to 8	none	4 to 14
457	98 to 103	♀	Dorsal	8, 9	none	8 to 13
"	104 to 105	♀	Dorsal	6, 7	25, 28	S
"	104[‡]	♀	Dorsal	3	none	6
"	105[‡]	♀	Dorsal	6	none	67
374	106, 107	♀	Dorsal	6, 7	25	S
274	108	♀	Anal	none	—	S
"	108[‡]	♀	Dorsal	8	none	62
"	109 to 125	♀, ♂	Both	?	none	8 to 11

[*] The numbers indicate the interval, in days, between transplantation and the onset of the sloughing process.

[**] The numbers indicate the interval, in days, between transplantation and the beginning of the regeneration process.

[†] S indicates successful fin transplants, which survived as long as their hosts. The numbers indicate in days the period before the disintegration of the transplanted fin.

[‡] Fish that received a second fin transplant.

In 2 of the 14 transplants no sloughing of fins took place. In the others the grafts appeared to be completely lost except for slight elevations at the points of their prior insertions. Three to 4 weeks later blastemas formed at the mouths of the pockets and, 4 weeks later, all grafts had regenerated. In 5 hosts, the regenerated fins were slightly deformed. This is not surprising because, in some, only parts of fins were transplanted and, in one, the fin graft was apparently inserted imperfectly. Histological study revealed that all fin grafts were well vascularized 3 months after they had been transplanted (FIGURE 3).

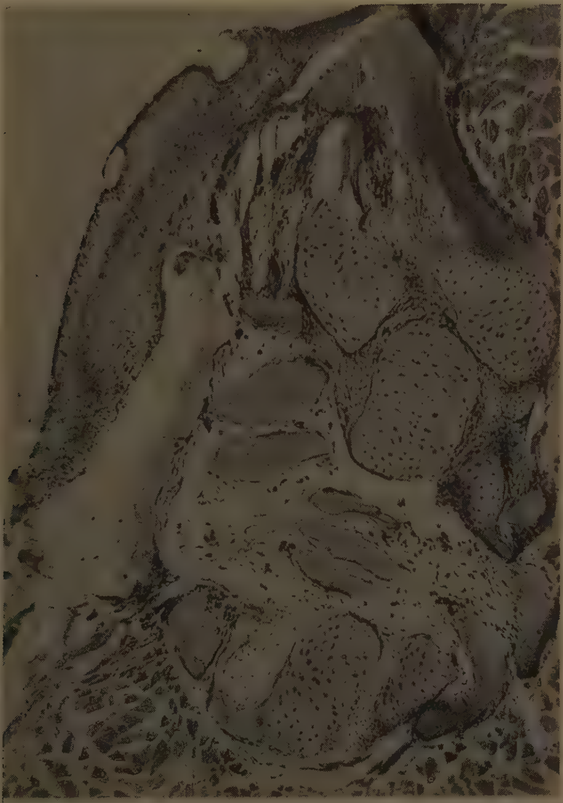

FIGURE 3. Cross section through the site of a successful isotransplant of a dorsal fin in a platyfish (strain 30^{17}) fixed three months after transplantation time. The basal rays of the transplanted fin are well imbedded and integrated in the myotomes of the host. Hematoxylin and eosin. ×100.

The dorsal fins of 6 donors used in the above transplantation experiments possessed erythrophores. When these 6 transplanted fins regenerated, after first having sloughed off, their erythrophores reappeared (TABLE 3, Nos. 1 to 6).

TABLE 3

FATE OF THE PIGMENT CELLS IN THE TRANSPLANTED FINS

Fish number	*Pigmentary genes* of*		*Pigment cells present in transplanted fin*	
	Host	*Donor*	*Originally*	*Three months after*
1 to 6	dr	dr	Erythrophores	Erythrophores
7 to 14	dr	+	None	None
27 to 29	+	r	Erythrophores	Erythrophores
106	+	dr	Erythrophores	Erythrophores
45 to 47	sd dr	+	None	None
51 to 55	sd dr	+	None	None
50	sd	+	None	None
48, 49	+	sd dr	Erythrophores, Macromelanophores	Erythorphores, Macromelanophores

**Genes*	*Names*	*Chromatophores*	*Position*
sd	Spotted-dorsal	Macromelanophores	Dorsal fin
dr	Dorsal-red	Erythrophores	Dorsal fin
r	Red	Erythrophores	Over-all
+	Multiple recessive	Micromelanophores only	Over-all

All fishes had micromelanophores in addition to their other chromatophores.

Homotransplants. (1) In the platyfish of the inbred strain 163, 11 of 13 transplantations of dorsal fins were successful (TABLES 1, 2). Two dorsal fins containing macromelanophores and erythrophores were successfully grafted into the caudal peduncles of 2 hosts that did not possess either of these chromatophores in their dorsal fins. Both types of pigment cells appeared in the 2 fins that regenerated (TABLE 3, Nos. 48, 49). In the reverse experiment dorsal fins that were free of these pigment cells were transplanted to hosts that had pigmented dorsal fins. Neither macromelanophores nor erythrophores appeared in the regenerated fins (TABLE 3, Nos. 45 to 47, 51 to 55).

(2) Each of the 4 fin transplants made between red members of the Bh^7 strain (TABLE 2, Nos. 30 to 33) was negative. One of the 4 transplants between members of the gray Bh^7 strain was successful. It did not pass through the characteristic sloughing process (TABLE 2, Nos. 34 to 37). Also, only 1 graft, out of 5, was successful between members of strain 492 (TABLE 2, Nos. 38 to 42).

(3) The 11 dorsal or anal fin transplantations that were made between members of pedigree 500 (TABLE 2, Nos. 15 to 25) first sloughed off but, after 3 to 4 weeks, 9 of the 11 regenerated.

(4) Four orange-yellow anal fins pigmented by xanthoerythrophores were removed from 4 females of pedigree 500 and were transplanted into the caudal peduncles of 4 sexually mature males of pedigree 501 (TABLE

2, Nos. 26 to 29). After the ninth day, the anal fin grafts in all 4 hosts had sloughed off, leaving only tiny spots of orange tissue. After 3 weeks, 3 anal fins began to regenerate and redevelop xanthoerythrophores (TABLE 3, Nos. 27 to 29). The fourth graft persisted for 7 weeks as a tiny speck of orange-colored tissue.

(5) Fifty-three transplants were made with sexually mature fish of strains 274, 374, 427, and 457 (TABLE 2, Nos. 56 to 108). Only 5 transplants were successful (TABLE 2, Nos. 104 to 108); in all but 1 of these (No. 108) the fins passed through the characteristic sloughing process, then regenerated after 3½ weeks. One of the transplanted dorsal fins that possessed erythrophores (TABLE 3, No. 106) regenerated a new dorsal fin that developed erythrophores. Three months after the first transplantation, each of these 5 fish received a second graft from a donor unrelated to the primary donor. Fish 105, 107, and 108 received dorsal fins removed from members of strain 274. Fish 104 and 106 were implanted with fins from members of strain 500. The implants in 106 and 107 became detached; the one in 104 sloughed off rapidly and disappeared completely on the sixth day. In fish 105 and 108, the lower third of the graft withstood the sloughing process for 2 months; then the secondary grafts finally disintegrated, but the primary fin grafts in all 5 fish remained unchanged (FIGURE 4).

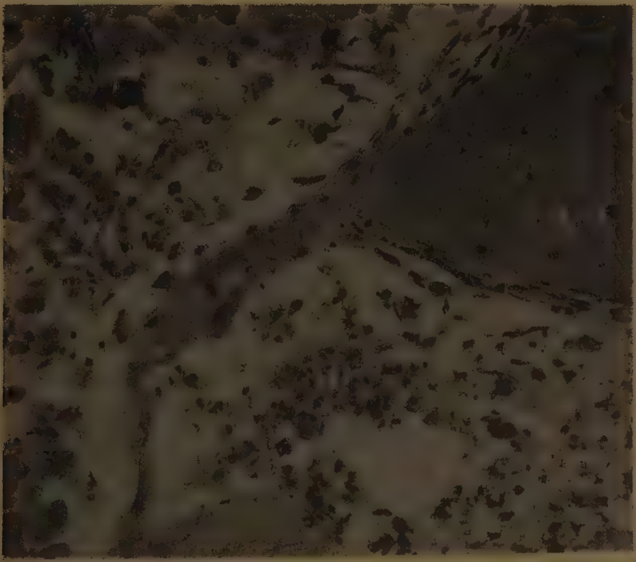

FIGURE 4. Cross section through a homotransplant of a dorsal fin in a platyfish (strain 427) eight days after transplantation. This transplant differs from the transplant in FIGURE 3 because large numbers of erythrocytes are scattered freely throughout the tissue spaces. The tissues surrounding the degenerating fin ray have been destroyed. Hematoxylin and eosin. ×215.

(6) In order to determine whether the age of the host influences the success of fin transplantation, 17 grafts were made between siblings of strain 274 which were less than 2 days old (TABLE 2, Nos. 109 to 125) (it is well to remember that platyfish are born about 21 days after fertilization of the eggs, according to Hopper (1943), so that a 2-day-old platyfish is actually in its twenty-third day of life). All grafts were found intact after 3 days but, after the eighth day, only traces of 11 grafts were seen. After 11 days none were left.

Discussion

Approximately one third of Held's autotransplants of dorsal and anal fins of adult *Xiphophorus maculatus* were successful, but all his homotransplants failed. In his opinion the failure of his homotransplants was due to the heterozygosity of the host and donor fish. Earlier, Goodrich and Nichols (1933) obtained somewhat comparable results when they attempted to transplant scales and their surrounding tissues in the goldfish. Their autotransplants were successful, but their homotransplants disintegrated. Other attempts at homotransplantation of various tissues in fish by Secerov (1912), Sauter (1934), and Nardi (1935) were failures.

In transplanting fins from one individual to another we used highly inbred strains of fish for the first time and were successful. Our results correspond to those of others who used highly inbred animals such as rats and mice. Close genetic correspondence between host and donor in adult *X. maculatus* is definitely of prime importance in the successful transplantation of fins from one animal to another. Fin transplantations between the members of our highly homozygous strain 30[17] were most successful. In transplants between members of strain 163, which had been inbred for fewer generations, the results were slightly less successful. The chances for successful homotransplants are enhanced when host and donor have at least one similar set of chromosomes; this was the case in our strains 500 and 501 (TABLE 1). In homotransplants between members of our heterozygous strains the number of successful grafts was small.

No sloughing occurred in only two isotransplanted and two homotransplanted fins. But usually, after a fin had been transplanted—and this applies to our iso- and homotransplants and Held's autotransplants—the sloughing process set in. In sloughing, after the transplanted fin had regained its vascularity, the blood vessels became distended, the blood flow stopped, the vessels ruptured, and blood entered the intercellular spaces. Since sloughing of fins occurred in all categories of fin transplants, it is unlikely that the degenerative process can be attributed to tissue antagonism. We are not prepared to compare the sloughing process in fin grafts to a somewhat similar process in the degeneration of skin grafts in mammals.

Within the limited scope of our experiments the age of the post-embryonic host did not affect the outcome of our transplantations. Recently Humm and Young (1956) reported some success in transplanting fragments of ectoderm, neural tube, and mesoderm of platyfish-swordtail hybrid embryos into pure swordtail host embryos.

The pigment cell patterns in the transplanted fin may disappear during the sloughing process, but they reappear in the regenerated fin essentially the same as before and are in no way affected by the pigmentary genes of the host (TABLE 3). In their experiments in transplanting scales in the goldfish, Goodrich and Nichols (1933) found that the pigment cells in the tissues surrounding the scales retained their identity in their new sites. Marcus and Gordon (1954) showed that transplanted melanoma cells in xiphophorin fishes recreated a state of melanosis in their new site. The size, shape, and polarity of transplanted fin rays and scales were maintained in fishes in the experiments that have been described by Birnie (1947) and Nardi (1935).

Summary

(1) Homo- and isotransplantations of anal and dorsal fins were made between members of various strains of platyfish, *Xiphophorus maculatus,* in order to study the role of genetic factors in transplantation.

(2) Nearly all transplanted fins sloughed off three to nine days after the operation. The degeneration began at the distal margin of the fin and progressed toward the base, stopping at various levels. Eventually a new fin regenerated, if the base of the transplanted fin remained intact.

(3) The results from the transplantation of anal and dorsal fins between members of highly inbred platyfish were extremely successful. Those from transplantation of fins between members of less highly inbred platyfish were less successful. Results from transplantation of fins between nonrelated platyfish were quite poor.

(4) The pigment cells of successfully transplanted and regenerated fins redeveloped in accordance with the donor's genetic constitution for fin coloration.

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Printed in the United States of America